EXHIBIT PP

Patent Docket P150C2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Shmuel Cabilly et al. Serial No.: 08/422,187 Filed: 13 April 1995 For: METHODS, VECTORS AND HOST CELLS FOR PRODUCING ANTIBODY POLYPEPTIDES (as amended)	Group Art Unit: 1644 Examiner: P. Gambel [redacted]

Declaration of Dr. Richard Axel

I, Dr. Richard Axel, do hereby declare and state that:

1. I am a citizen and resident of the United States of America. I am a paid member of the Scientific Advisory Board Of Genentech, Inc., whom I understand to be an assignee of the above application. I have substantial experience and expertise in the field of recombinant DNA technology. My curriculum vitae is attached as Exhibit A. I was active in the field of recombinant DNA technology in 1983. As of that time, the general level of skill in that field was a Ph. D. in molecular biology or similar discipline, and about two years of experience consistent with that level of education.

2. I have reviewed the specification and claims of the above application. The specification teaches how to make an antibody heavy chain, light chain (or fragment of a heavy chain or light chain) which has specificity for a desired antigen and comprises a human constant region and a variable region comprising non human mammalian variable region sequences, using a variety of different host cells and recombinant vectors, as well as various expression and recovery methods. While the Examples on pages 30-53 of the application describe production of the recombinant antibody or

antibody fragment in *E. coli* host cells with recovery of individual immunoglobulin chains and reconstitution of an antibody capable of specifically binding antigen, the patent specification describes in sufficient detail other host cells, recombinant vectors, and methods for making the claimed antibodies and antibody fragments such that a person skilled in recombinant DNA technology as of 1983 could have made antibodies or antibody fragments by other methods.

3. Aside from prokaryotic host cells such as *E. coli*, the application explains that eukaryotic microbes, such as yeast, may also be used. *Saccharomyces cerevisiae* is specifically mentioned and recombinant expression vectors compatible with these yeast hosts are described (see pages 16-17). Wood *et al. Nature* 314: 446-449 (1985) (Exhibit B attached) confirms that *Saccharomyces cerevisiae* is a suitable host cell for producing an antibody capable of specifically binding a desired antigen. In column 2 on page 448, Wood *et al.* explain that "extracts of cells transformed with both plasmids, and expressing both light and heavy chains, showed a strong, specific signal similar to that of the hybridoma B1-8 protein." The above application describes coexpression of light and heavy chains in the same host (see, for example, page 23, last paragraph and page 43, last paragraph) *e g.* in *Saccharomyces cerevisiae* (see line 35 on page 16). Thus, based on the application, the ordinarily skilled molecular biologist in 1983 could have made an antibody or antibody fragment in yeast and that antibody or fragment could have been used, among other things, to detect a desired antigen.

4. The specification explains that, as an alternative to *in vitro* reconstitution, reconstitution of the antibody or antibody fragment may be achieved *in vivo* in a microorganism which secretes the IgG chains from the reducing environment of the cytoplasm (page 23,

lines 32-33), *e.g.* into the periplasmic space of a gram negative bacteria such as *E. coli* (page 23, lines 21-25). This *E. coli* secretion strategy was indeed adopted by others such as Better *et al.*, *Science* 240:1041-1043 (1988) (Exhibit C attached) as well as Skerra and Pluckthun *Science* 240:1038-1041 (1988) (Exhibit D attached) and found to result in the production of antibody fragments capable of specifically binding a desired antigen. Antibody fragments are disclosed in the application; see page 13, line 24 through to page 14, line 22; page 30, lines 8-14, and page 52, line 12 through to line 34 on page 53. Skerra and Pluckthun used *omp*A and *pho*A signal sequences available in 1983; see Fig. 1 of Exhibit D which references pre-1983 publications Movva *et al. J. Biol. Chem.* 255:27-29 (1980) (Exhibit E attached) and Inouye *et al. J. Bacteriol.* 149:434-439 (1982) (Exhibit F attached). Therefore, the specification sufficiently describes production of antibodies or antibody fragments by methods which do not involve an *in vitro* reconstitution step.

5. Aside from the production of antibodies or antibody fragments in prokaryotes and eukaryotic microbes, the application describes the production of antibodies or antibody fragments in a variety of alternative eukaryotic host cells. In particular, page 18, first paragraph of the application explains that tissue cultures derived from multicellular organisms were suitable expression hosts. Methods for propagation of vertebrate cells in cell culture were known as early as 1973 (*Tissue Culture*, Academic Press, Kruse and Patterson, editors, (1973); cited on page 18 of the patent application). A large variety of suitable mammalian host cells were available in 1983, including VERO, HeLa, Chinese Hamster Ovary (CHO), W138, BHK, COS-7 and MDCK cells specifically mentioned on page 18 of the application. The COS-7 line of monkey cells was described by Gluzman, *Cell* 23:175-182 (1981) (Exhibit G attached)

and CHO-K1 cells could have been obtained from the American Type Culture Collection (ATCC CCL61).

6. In 1983, one could have made the antibody or antibody fragment in COS-7 cells, for example, based on the disclosure of the application. The application specifically describes the use of COS-7 cells at page 18, lines 8-10. The application further indicates in the last two paragraphs on page 18 that vectors with Simian Virus 40 (SV40) promoters and origin of replication are particularly effective as expression vectors when using mammalian host cells. Transfection of COS-7 cells with such SV40-derived expression vectors and expression of recombinant protein by the transfected COS-7 cells was described on pages 24-26 of EP 0 073 656, published March 9, 1983 (Exhibit H attached). Based on the teachings of the above application concerning expression of antibodies and antibody fragments combined with the disclosure in Exhibit H, I believe that the ordinarily skilled molecular biologist in 1983 could have made functional antibodies or antibody fragments in COS-7 cells.

7. The antibody or antibody fragment could also have been expressed by a CHO cell. US Patent No. 4,399,216 which issued August 16, 1983 (Exhibit I attached) describes the use of the dihydrofolate reductase (DHFR) selection gene and exposure to methotrexate for the purpose of amplifying expression of eukaryotic genes in CHO cells (see, column 7, line 3 through to column 9, line 53 and, in particular, column 8, lines 42-48). I am a coinventor of this patent. The techniques in Exhibit I, coupled with the teachings in the above patent application, would have enabled one of ordinary skill in the field of recombinant DNA technology to express antibodies or antibody fragments in CHO cells, no later than 1983, using only standard methods and without exercising

inventive skill. As further evidence of the level of skill in the field as of 1983, attached as Exhibit J is US Patent No. 4,965,196, which I understand contains a specification originally filed in 1983. Exhibit J describes expression of recombinant proteins in CHO cells using DHFR selection and methotrexate to amplify expression. See, Examples 4-7 in columns 8-10. The effective use of DHFR selection and methotrexate amplification is also described in detail at column 5, line 32 through to column 6, line 8.

8. Therefore, for the reasons given above, I believe that the ordinarily skilled molecular biologist in 1983 could have made antibodies or antibody fragments in a variety of different host cells using various recombinant expression vectors and methodologies without excessive experimentation, based on the disclosure of the above application.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date 8/26/99 Richard Axel Richard Axel